Jul 11th, 2:45 PM - 3:45 PM

Examining Preservation Methods for Long-Term Fecal Matter Storage

Caroline Veronica Ruiz
Elizabethtown College, ruizc@etown.edu

Celeste Workman
Elizabethtown College, workmanc@etown.edu

Examining Preservation Methods for Long-Term Fecal Matter Storage

Celeste Workman, Caroline Veronica Ruiz, and Debra Wohl
Department of Biology at Elizabethtown College, Elizabethtown, PA

Aims

- To analyze metabolic activity of bacteria after storage in order to determine which cryogenic technique best preserves fecal matter samples
- Personalize fecal matter transplant (FMT) process

Introduction

The human microbiota contains many diverse species of bacteria and other microorganisms. Disruption of the microbiome within the gastrointestinal tract can lead to the proliferation of harmful bacteria such as vancomycin-resistant *Enterococcus* (VRE) or *Clostridium difficile*[1]. *Clostridium difficile,* or *C. diff,* is a common hospital-acquired pathogen which disrupts the normal function of healthy bacteria[2]. Though typically treated with antibiotics, *C. diff* is often able to resurface due to antibiotic overuse.

Since antibiotics may be ineffective when treating bacterial diseases such as *C. diff* other treatments are needed to eliminate the threat of infection and reestablish the healthy microbiota in the gut. Fecal matter transplants (FMTs) have been used to successfully treat patients in clinical studies. However, using donor samples can put patients at risk and has resulted in at least one death reported by the U.S. Food and Drug Administration[3]. By personalizing the donation process of fecal samples, the risk of introducing harmful microorganisms could potentially be reduced.

Future Studies

- Use DNA sequencing to determine which microorganisms are lost after storage and which metabolic activities are impacted as a result

Materials and Methods

Original Sample
- 80g dog fecal sample partitioned into two parts
- 40g mixed with120 mL 0.85% NaCl (saline)
- 40g mixed with 120 mL H_2O (water)

Homogenization

Day 0 | **Day 21**

Dilutions
- A (10^{-1}), B (10^{-3}), C (10^{-5})
- NaCl or H_2O as diluents

Biolog™ EcoPlates
- Aerobic and anaerobic environments created during incubation
- Incubated at 37°C for ~36 hrs

Data Analysis
- Color change in Biolog™ plates used to compare bacterial function at Day 0 and Day 21 after -80°C storage

Fig. 1 Flowchart of homogenization and dilution procedure

Table 1. Sample Groups used on Day 0 (Before storage) and Day 21 (After storage)

Sample Groups	
Day 0 (Before)	**Day 21 (After)**
Water	Water glycerol (WG)
	Water (WO)
Saline	Saline glycerol (SG)
	Saline (SO)

Acknowledgments

- Funding provided by the SCARP program
- Elizabethtown College Biology Department
- Fecal samples provided by Dr. Bridge's dogs
- Preliminary research by Cecelia Martin and Yanellis Bonano

Results

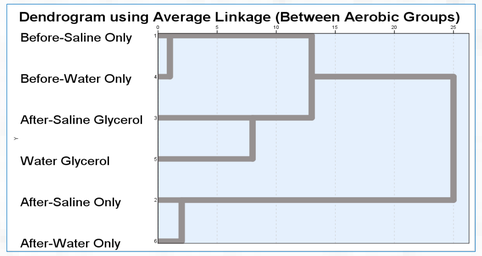

Fig 2. Dendrogram comparing similar metabolic function between before and after samples in aerobic environments

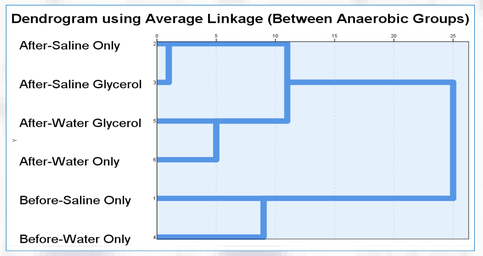

Fig 3. Dendrogram comparing similar metabolic function between before and after samples in anaerobic environments

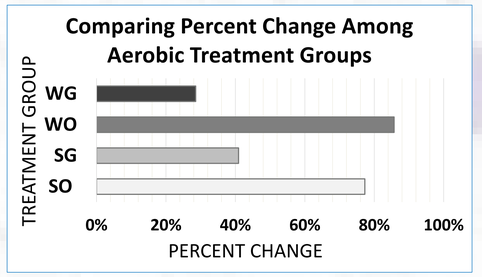

Fig 4. Percent change among aerobic treatment groups calculated after storage at -80°C

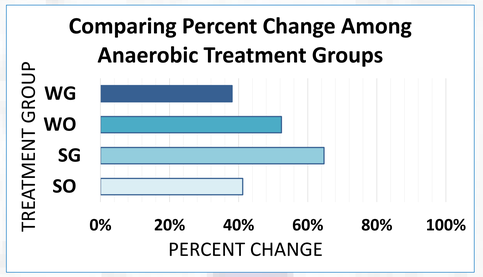

Fig 5. Percent change among anaerobic treatment groups calculated after storage at -80°C

Discussion and Conclusion

- After calculating percent change of metabolic activity among treatment groups, all treatment groups showed loss of cell function after storage
- The water glycerol treatment group had the smallest percent change out of all other treatment groups
- Treatment groups that contained glycerol and were incubated in an aerobic environment more closely resembled the metabolic activity seen in original samples
- Treatment groups with no glycerol lost the most metabolic activity after storage
- **Fecal matter stored with glycerol preserves microorganism communities better than treatments with no glycerol**
- This study of metabolic activity and cell viability supports earlier findings based on DNA preservation

References
1. Taur, Y. et al. Reconstitution of the gut microbiota of antibiotic-treated patients by autologous fecal microbiota transplant. Sci. Transl. Med 10, eaap9489 (2018)
2. Centers for Disease Control and Prevention (2018). *Clostridioidium difficile (C. diff)* https://www.cdc.gov/cdiff/what-is.html
3. United States Food and Drug Administration (2019). FDA In Brief: FDA warns about potential risk of serious infections caused by multi-drug resistant organisms related to the investigational use of Fecal Microbiota for Transplantation
4. https://www.biolog.com/wp-content/uploads/2018/06/MicroplateOnly_LR_RGB-e1529699504599.jpg